

1 Claims

- 2
- 3 1. A human embryonic stem cell line
- 4 characterised by at least one of the
- 5 following:
- 6 i) presence of the cell surface markers TRA-
- 7 1-60, GTCM2, and SSEA-4;
- 8 ii) expression of *Oct-4*;
- 9 iii) expression of *NANOG*;
- 10 iv) expression of *REX-1*; and/or
- 11 expression of *TERT*.
- 12
- 13 2. The human stem cell line as claimed in Claim
- 14 1 having two or more of the characteristics
- 15 i) to v).
- 16
- 17 3. The human stem cell line as claimed in Claim
- 18 2 having three or more of the characteristics
- 19 i) to v).
- 20
- 21 4. The human stem cell line as claimed in Claim
- 22 3 having four of the characteristics i) to
- 23 v).
- 24
- 25 5. The human stem cell line as claimed in Claim
- 26 4 having all of the characteristics i) to v).
- 27
- 28 6. The stem cell line hES-NCL1 deposited at
- 29 NIBSC under Accession No. P-05-001.
- 30
- 31 7. An embryonic stem cell bank comprising a
- 32 multiplicity of genetically distinct stem

1 cell lines as claimed in any one of Claims 1
2 to 6.

3

4 8. A method of screening an agent for toxicity
5 and/or for therapeutic efficacy, said method
6 comprising:

7 i. exposing a stem cell line as claimed in
8 any one of Claims 1 to 6 to said agent;

9 ii. monitoring any alteration in viability
10 and/or metabolism of said stem cells; and

11 iii. determining any toxic or therapeutic
12 effect of said agent.

13

14 9. A method of screening an agent for toxicity
15 and/or for therapeutic efficacy, said method
16 comprising:

17 i. exposing an embryonic stem cell bank as
18 claimed in Claim 7 to said agent;

19 ii. monitoring any alteration in viability
20 and/or metabolism of said stem cells;
21 and

22 iii. determining any toxic or therapeutic
23 effect of said agent.

24

25 10. A method of producing fibroblast-like cells,
26 said method comprising:

27 i. providing a stem cell line as claimed in
28 any one of Claims 1 to 6;

29 ii. allowing cells of said stem cell line to
30 differentiate into stem cell derived
31 fibroblast-like cells.

32

- 1 11. The method of Claim 10 which is conducted
2 without use of a specific stimulant for
3 differentiation.
4
- 5 12. The method as claimed in either one of Claims
6 10 and 11 wherein the fibroblast-like cells
7 are produced for a therapeutic purpose.
8
- 9 13. A method of culturing cells wherein the
10 fibroblast-like cells obtained as claimed in
11 Claims 10 or 11 act as feeder cells or
12 condition cell culture media used during
13 culture of the cells.
14
- 15 14. The method as claimed in Claim 13 wherein the
16 cells being cultured are stem cells.
17
- 18 15. A method of maintaining the viability of eggs
19 prior to or during fertilisation, wherein the
20 fibroblast-like cells obtained as claimed in
21 Claims 10 or 11 act as feeder cells or
22 condition cell culture media used during
23 maintenance of the eggs.
24
- 25 16. A method of culturing a blastocyst or embryo
26 prior to implantation into a receptive
27 female, wherein the fibroblast-like cells
28 obtained as claimed in Claims 10 or 11 act as
29 feeder cells or condition cell culture media
30 used during culture of the blastocyst or
31 embryo.
32

- 1 17. The fibroblast-like cell line hESCdF-NCL as
2 deposited at ECACC under Accession No.
3 04010601.
4
- 5 18. A method of culturing cells wherein hESCdF-
6 NCL cells act as feeder cells or condition
7 cell culture media used during culture of the
8 cells.
9
- 10 19. The method as claimed in Claim 18 wherein the
11 cells being cultured are stem cells.
12
- 13 20. A method of maintaining the viability of eggs
14 prior to or during fertilisation, wherein
15 hESCdF-NCL cells act as feeder cells or
16 condition cell culture media used during
17 maintenance of the eggs.
18
- 19 21. A method of culturing a blastocyst or embryo
20 prior to implantation into a receptive
21 female, wherein hESCdF-NCL cells act as
22 feeder cells or condition cell culture media
23 used during culture of the blastocyst or
24 embryo.
25
- 26 22. A self-feeder system for the growth of
27 undifferentiated stem cells, said system
28 comprising:
29 i. culturing a stem cell line as claimed in
30 any one of Claims 1 to 6; and
31 ii. and allowing some of the cells of said
32 stem cell line to differentiate into

1 stem cell derived fibroblast-like cells
2 whilst the remainder of the cells of
3 said embryonic stem cell line remain in
4 an undifferentiated pluripotent,
5 multipotent or unipotent state, whereby
6 said stem cell derived fibroblast-like
7 cells act as autogeneic feeder cells for
8 said stem cells.

9
10 23. A method of culturing a blastocyst, said
11 method comprising exposing said blastocyst
12 for a period of at least 12 hours to Buffalo
13 rat liver cells or to media conditioned by
14 Buffalo rat liver cells.

15
16 24. The method as claimed in Claim 23 wherein the
17 period of exposure is at least 48 hours.

18
19 25. The method as claimed in either one of Claims
20 23 and 24 wherein the period of exposure of
21 said blastocyst to said Buffalo rat liver
22 cells or to media conditioned by said Buffalo
23 rat liver cells immediately precedes
24 extraction of ICM cells from the blastocyst.

25
26 26. The method as claimed in any one of Claims 23
27 to 25 wherein the media conditioned by
28 Buffalo rat liver cells is produced by:
29 i. culturing at least 75000 Buffalo rat
30 liver cells/cm² in Glasgow medium for 24
31 to 36 hours; and

1 ii. recovering the media by removal of the
2 cells.

3
4 27. The method as claimed in any one of Claims 23
5 to 26 wherein the blastocyst can be cultured
6 to day 8 after fertilisation and retain
7 pluripotency.

8
9 28. The method as claimed in any one of Claims 23
10 to 27 wherein said blastocyst is a primate
11 blastocyst.

12
13 29. The method as claimed in Claim 28 wherein
14 said blastocyst is a human blastocyst.

15
16 30. A method for culturing a blastocyst, as
17 claimed in any one of Claims 23 to 29, said
18 method comprising:

19 i. culturing said blastocyst from
20 fertilisation in G1 media;
21 ii. transferring said blastocyst of step
22 i) to G2.3 media and maintaining said
23 blastocyst in the G2.3 media; and
24 iii. transferring said blastocyst of step
25 ii) to cell culture media conditioned
26 by Buffalo rat liver cells.

27
28 31. The method as claimed in Claim 30 wherein the
29 blastocyst is cultured in the conditions of
30 step i. for 1 to 3 days.

31

- 1 32. The method as claimed in either one of Claims
2 30 and 31 wherein the blastocyst is cultured
3 in the conditions of step ii. for 2 to 3
4 days.
5
- 6 33. The method as claimed in any one of Claims 30
7 to 32 wherein the blastocyst is cultured in
8 the conditions of step iii. for 1 to 3 days.
9
- 10 34. The method as claimed in any one of Claims 30
11 to 33 wherein the cell culture media is
12 Dulbecco's modified Eagle's medium optionally
13 supplemented with 15% (v/v) Glasgow medium
14 and conditioned by Buffalo rat liver cells.
15
- 16 35. A method of *in vitro* fertilisation, said
17 method comprising culturing a blastocyst as
18 claimed in any one of Claims 23 to 34; and
19 implanting said cultured blastocyst into a
20 receptive female.
21
- 22 36. A method of producing an embryonic stem cell
23 line, said method comprising:
24 i. culturing a blastocyst as claimed in any
25 one of Claims 23 to 34; and
26 ii. extracting cells of the inner cell mass
27 (ICM) from said blastocyst and culturing
28 the cells to produce an embryonic stem
29 cell line therefrom.
30

1 37. The method as claimed in Claim 36 wherein
2 said stem cell line is a primate embryonic
3 stem cell line.

4
5 38. The method as claimed in Claim 37 wherein
6 said stem cell line is a non-human primate
7 embryonic stem cell line.

8
9 39. The method as claimed in Claim 37 wherein
10 said stem cell line is a human embryonic stem
11 cell line.

12
13 40. The method as claimed in any one of Claims 36
14 to 38 wherein said embryonic stem cell line
15 is a pluripotent stem cell line.

16
17 41. A self-feeder system for the growth of
18 undifferentiated stem cells, said system
19 comprising:
20 i. culturing a blastocyst as claimed in
21 Claims 23 to 34;
22 ii. extracting cells of the ICM from said
23 blastocyst and culturing the cells to
24 produce an embryonic stem cell line
25 therefrom; and
26 iii. and allowing some of the cells of said
27 embryonic stem cell line to differentiate
28 into stem cell derived fibroblast-like
29 cells whilst the remainder of the cells
30 of said embryonic stem cell line remain
31 in an undifferentiated pluripotent,
32 multipotent or unipotent state, whereby

1 said stem cell derived fibroblast-like
2 cells act as autogeneic feeder cells for
3 said stem cells.

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5 42. An embryonic stem cell bank comprising a
6 multiplicity of genetically distinct stem
7 cell lines obtained by the method as claimed
8 in any one of Claims 36 to 39.

9

10 43. A method of producing fibroblast-like cells,
11 said method comprising:

12 i. culturing a blastocyst as claimed in any
13 one of Claims 23 to 34;

14 ii. extracting cells of the ICM from said
15 blastocyst and culturing the cells to
16 produce an embryonic stem cell line
17 therefrom; and

18 iii. allowing cells of said embryonic stem
19 cell line to differentiate into stem cell
20 derived fibroblast-like cells.

21

22 44. A method of culturing cells wherein the
23 fibroblast-like cells obtained by the method
24 of Claim 43 act as feeder cells or condition
25 cell culture media used during culture of the
26 cells.

27

28 45. A method of maintaining the viability of eggs
29 prior to or during fertilisation wherein the
30 fibroblast-like cells obtained by the method
31 of Claim 43 act as feeder cells or condition

1 cell culture media used during maintenance of
2 the eggs.

3

4 46. A method of a blastocyst or embryo prior to
5 implantation into a receptive female wherein
6 the fibroblast-like cells obtained by the
7 method of Claim 43 act as feeder cells or
8 condition cell culture media used during
9 culture of blastocyst or embryo.

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